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Total seed oil and fatty acid methyl ester contents of Cuphea accessions

Winthrop B. Phippen [a,*], Terry A. Isbell [b], Mary E. Phippen [a]

[a] *Agriculture Department, Western Illinois University, Macomb, IL 61455, USA*
[b] *New Crops Processing and Technology Research Unit, National Center for Agricultural Crops Utilization Research, USDA, ARS, Peoria, IL 61604, USA*

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Abstract

Many Cuphea species have been identified as potential new sources of unique fatty acids for both the lubricant and soap and detergent industries. Current breeding programs have focused on improving agronomic traits to make Cuphea suitable for commercial production. Breeding programs are now focusing on altering total oil and fatty acid content with a special interest in lauric and capric acids. Accessions identified as high in oil content and rich in single fatty acids will be introgressed into the current agronomically sound breeding lines. The objective of this study was to develop a reliable and efficient method for evaluating Cuphea accessions for their total oil and fatty acid content in Cuphea seed. One hundred and eighty-five accessions of Cuphea were screened for their total oil and fatty acid content. Total oil content was determined by nondestructive pulsed NMR on whole Cuphea seed. Previous extraction and derivatization procedures were combined and optimized to minimize time and complexity in extracting medium-chain triglycerides and derivatizing them into fatty acid methyl esters for gas chromatographic analysis. Extraction and derivatization procedures were validated for linearity, precision, accuracy, and sample stability.

Total oil content ranged from 10.1% in *Cuphea llavea* to 39.5% in *Cuphea wrightii* var. *wrightii* with a 2.5% relative standard deviation. *C. llavea* had the highest levels of capric acid at 92.0%. The highest levels of lauric acid were present in *C. wrightii* var. *wrightii* at 72.8%. Samples were stable for 24 h at room temperature. Recoveries for methyl caprate and methyl laurate were 98%. Relative standard deviations for methyl caprate and methyl laurate were 2.9% and 6.1%, respectively. Validation results demonstrated that the extraction, derivatization, and gas chromatographic analysis produced reliable and reproducible results.

The Cuphea species identified in this study can serve as potential new sources for high seed oil content and fatty acids to be introduced into the current advanced breeding lines.

Keywords: *Cuphea* sp.; Gas chromatography; Seed oil; Fatty acid methyl ester

1. Introduction

Temperate plant species whose seed oils are rich in medium-chain fatty acids (MCFAs) are relatively rare (Wolf et al., 1983). One genus of particular interest is Cuphea, grown as a temperate annual oilseed crop with high levels of MCFAs such as capric and lauric acid (Graham et al., 1981; Graham and Kleiman, 1985, 1992). These fatty acids are important to the chemical industry for the manufacturing of detergents, surfactants, lubricants, and other products (Wolf et al., 1983; Thompson et al., 1990; Cermak and Isbell, 2004). Currently, the U.S. soap and detergent industry receives

* Corresponding author. Tel.: +1 309 298 1251; fax: +1 309 298 2280.
E-mail address: wb-phippen@wiu.edu (W.B. Phippen).

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one-half of these fatty acids from the petroleum industry and the other half from imported coconut and palm kernel oils.

Studies conducted in the early 1980s on Cuphea focused primarily on characterizing the fatty acid profiles of various species collected from the wild (Graham et al., 1981; Wolf et al., 1983; Hirsinger, 1985). These studies helped direct early breeding programs to focus on the lauric acid-accumulating species, *Cuphea wrightii* for the soap and detergent industry (Thompson et al., 1990). Unfortunately, domesticating this species proved to be extremely difficult due to seed shattering, open pollination, and poor agronomic traits. More recent efforts with the high capric acid species *Cuphea viscosissima* and *Cuphea lanceolata* have been successful in improving many of the agronomic traits necessary for Cuphea domestication (Knapp, 1993). However, non-shattering and determinacy still pose difficult hurdles. With efforts continuing to improve agronomic traits, altering total oil and fatty acid content will soon become a priority.

The Cuphea germplasm collection is currently maintained at the USDA Plant Introduction Station in Ames, IA. Several studies have screened portions of the collection under diverse protocols and conditions. Extraction method, location and time of seed production, and differing analytical conditions have all lead to variations in total oil and fatty acid composition being reported for individual accessions. Many of the early studies often involved complicated and difficult extraction and analytical procedures requiring hours of preparation. These methods are impractical for supporting high throughput breeding programs aimed at developing new varieties. The objective of this study was to determine total oil and fatty acid content of a subset of the available USDA Cuphea germplasm.

2. Materials and methods

2.1. Materials

Cuphea seed: One hundred and eighty-five accessions of Cuphea were obtained from the North Central Regional Plant Introduction Station in Ames, Iowa. PSR23 seed (*C. viscosissima* × *C. lanceolata*) was obtained from the fall 2003 Cuphea harvest at Western Illinois University, Macomb, IL.

Reagents: Potassium hydroxide (KOH) pellets, 95% sulfuric acid (H_2SO_4), and Optima^{TM} grade hexane and methanol were purchased from Fisher Scientific (Fair Lawn, NJ).

Mixing solutions: A 1 M sulfuric acid solution in methanol was made by slowly adding 5.6 ml of 95% H_2SO_4 to a 100 ml class A volumetric flask partially filled with methanol. The solution was cooled to room temperature and then diluted with methanol to a final volume of 100 ml. A 0.5 M KOH solution was made by adding 1.7 g of KOH pellets to 50 ml of methanol and vortexing to dissolve the pellets. Both solutions were made fresh every 7 days.

2.2. Total oil content in whole Cuphea seed

The total oil content was determined by nondestructive, low-resolution pulsed NMR (Bruker Minispec PC 120, 180-mm absolute probehead) on whole Cuphea seed. Seed samples ranged from 0.1 to 1 g from each accession. Final readings were derived from an average of 25 pulses. A calibration curve from 0.03 to 1 g was prepared from weighed samples of PSR23 Cuphea oil suspended on tissue material. Precision of the method was tested by analyzing six 0.1 g samples of PSR23 seed for their oil content and calculating the relative standard deviation.

2.3. Fatty acid content in Cuphea seed

Extraction and derivatization procedure: Medium-chain triglycerides were extracted and derivatized into fatty acid methyl esters in a three-step hydrolysis and methylation procedure. Seed samples (100 mg) were placed in 16 mm × 100 mm screw cap tubes with 2 ml of 0.5 M KOH in methanol. Seeds were ground with a Teflon stirring rod for 20 s in the tube, capped, and incubated at 60 °C for 1 h in a dry heat block. Two milliliters of 1 M H_2SO_4 in methanol was added and incubated at 60 °C for an additional 15 min. Following the two incu-

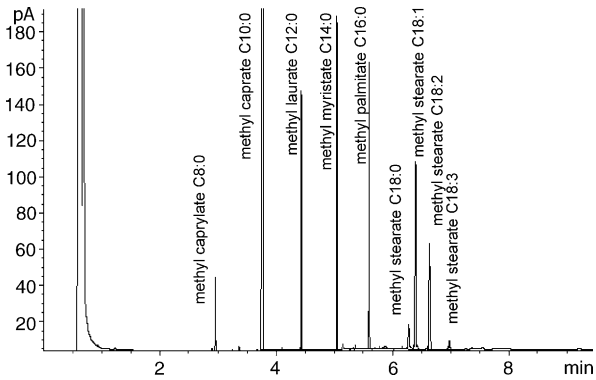

Fig. 1. Gas chromatographic profile of seed oil constituents in PSR23 Cuphea seed.

Table 1
Total oil and fatty acid methyl ester contents (%) for 60 accessions of *C. lanceolata*

Identifier	Total oil	C8	C10	C12	C14	C16	C18:0	C18:1	C18:2	C18:3
PI 594936	33.13	2.44	91.08	1.22	0.60	1.12	0.36	1.35	1.83	–
PI 594958	33.08	1.07	87.88	2.02	1.74	2.26	0.18	2.09	2.76	–
PI 561501	32.61	0.95	87.46	1.86	1.72	2.32	0.27	2.69	2.73	–
PI 596739	31.85	1.02	88.22	1.99	1.63	2.05	0.55	2.02	2.52	–
PI 594940	31.29	1.11	89.27	1.86	1.22	1.76	0.34	1.67	2.77	–
PI 594943	31.16	0.41	80.59	4.06	4.32	3.15	0.36	3.62	3.48	–
PI 534848	31.12	0.97	88.98	1.98	1.37	1.79	0.27	1.78	2.84	–
PI 594948	30.88	0.22	80.98	4.01	3.99	2.92	0.69	4.31	2.87	–
PI 594939	30.79	0.80	87.36	1.78	1.19	2.04	0.52	2.45	3.74	0.11
PI 534868	30.75	0.95	87.11	2.03	1.52	2.21	0.46	2.43	3.28	–
PI 534864	30.71	0.94	88.88	1.80	1.35	1.88	0.49	1.98	2.68	–
PI 561486	30.70	0.45	79.60	[4.29]	4.94	3.37	0.43	3.65	3.27	–
PI 594935	30.67	1.13	89.08	1.85	1.17	1.76	0.41	1.61	2.98	–
PI 594937	30.64	1.16	90.03	1.81	1.23	1.61	–	1.55	2.61	–
PI 594946	30.63	0.88	87.70	1.89	1.28	2.00	0.54	2.09	3.61	–
PI 596738	30.36	0.47	82.21	3.98	4.33	2.70	0.78	3.09	2.44	–
PI 534857	30.08	1.00	88.69	1.87	1.41	2.02	–	2.06	2.94	–
PI 534858	29.98	0.88	87.37	1.74	1.47	2.36	0.16	2.85	3.18	–
PI 594957	29.98	0.69	89.42	2.03	1.54	1.96	–	1.89	2.47	–
PI 534865	29.93	0.97	87.48	2.07	1.55	2.07	0.56	2.27	3.03	–
PI 594941	29.89	1.11	89.15	1.95	1.26	1.80	–	1.91	2.80	–
PI 534869	29.76	1.03	88.71	1.81	1.52	2.03	0.20	1.86	2.85	–
PI 534830	29.67	1.02	89.38	2.49	1.37	1.60	0.15	1.63	2.37	–
PI 594930	29.59	0.88	86.59	2.21	1.75	2.38	0.42	2.05	3.72	–
PI 594942	29.56	1.03	89.09	1.94	1.36	1.84	–	1.96	2.78	–
PI 561485	29.36	1.10	90.00	1.78	1.40	1.71	0.15	1.53	2.35	–
PI 534861	29.24	0.99	87.51	1.89	1.51	2.22	0.54	1.97	3.37	–
PI 594934	28.96	0.99	85.59	2.29	1.91	2.39	0.52	2.39	3.81	0.11
PI 534870	28.87	0.97	89.56	1.84	1.32	1.81	–	1.89	2.60	–
PI 594944	28.54	0.66	80.14	4.14	4.47	3.19	0.54	3.62	3.24	–
PI 534828	28.47	1.16	89.65	1.85	1.30	1.40	1.42	1.31	1.91	–
PI 534833	28.33	0.83	81.45	2.25	2.10	3.62	0.80	4.05	4.79	0.11
PI 594938	28.27	0.99	89.32	1.87	1.38	1.86	–	1.69	2.87	–
PI 594945	28.20	0.83	86.12	1.98	1.55	2.38	0.57	2.60	3.97	–
PI 534853	27.86	0.98	[91.39]	1.92	1.17	1.28	–	1.28	1.98	–
PI 596737	27.69	0.39	77.75	4.26	5.10	3.80	0.43	4.37	3.90	–
PI 534855	27.68	0.92	86.80	2.13	1.64	2.26	0.50	2.63	3.12	–
PI 534849	27.58	0.97	86.52	2.14	1.72	2.29	0.49	2.36	3.51	–
PI 534859	27.08	0.91	85.62	2.02	1.75	2.68	0.52	2.63	3.87	–
PI 534871	26.83	0.88	88.13	2.22	1.58	2.04	–	2.34	2.80	–
PI 594949	26.74	0.51	83.90	3.02	2.36	2.79	0.43	3.38	3.61	–
PI 596740	26.01	1.02	88.21	2.08	1.63	2.2	–	1.99	2.87	–
PI 534860	25.96	0.93	87.63	2.01	1.53	2.12	0.54	2.48	2.76	–
PI 534866	25.83	0.97	88.31	1.82	1.50	2.16	–	2.03	3.19	–
PI 534854	25.82	0.74	89.56	2.12	1.25	1.60	0.16	1.65	2.92	–
PI 534856	25.52	1.32	86.33	2.08	1.81	2.52	0.59	2.19	3.16	–
PI 534862	25.50	1.49	85.92	2.02	1.78	2.54	0.39	2.23	3.63	–
PI 594947	25.29	0.41	78.93	4.00	4.36	3.48	0.22	4.35	4.25	–
PI 534831	25.24	1.52	87.00	2.23	1.54	2.00	0.39	2.17	3.16	–
PI 534847	25.01	0.86	88.07	2.26	1.65	1.86	0.63	1.92	2.75	–
PI 534863	24.95	0.91	87.57	2.06	1.54	2.15	0.42	2.21	3.15	–
PI 534851	24.91	0.96	89.62	1.97	1.47	1.71	0.21	1.55	2.50	–
PI 534829	24.58	3.60	87.96	2.05	1.17	1.41	0.15	1.41	2.25	–
PI 534852	23.84	0.24	90.02	2.14	1.55	1.80	–	1.49	2.77	–
PI 534732	22.83	1.01	88.84	1.97	1.49	1.90	–	1.84	2.95	–
PI 594931	22.21	0.88	84.10	2.21	1.85	2.92	0.81	2.74	4.32	0.18
PI 534867	21.50	1.04	86.06	1.90	1.80	2.75	0.20	2.45	3.79	–
PI 534733	20.80	0.71	84.85	2.10	1.66	2.74	0.41	2.61	4.47	0.45
PI 534850	20.08	0.77	82.73	2.19	1.82	3.11	0.85	3.99	4.54	–

Bracketed numbers represent the highest recorded methyl caprate and methyl laurate levels for the species.

bation steps, 2 ml of H_2O and 5 ml of hexane were added to the tube, vortexed, and the layers were allowed to separate. The hexane layer was removed with a glass pipette and transferred into a gas chromatographic vial.

Gas chromatography analysis: Fatty acid methyl esters were analyzed using an Agilent 6890 gas chromatograph with a flame ionization detector (FID) and Agilent autosampler/injector. Analyses were conducted on a J&W brand DB-23 15 m × 0.25 mm i.d., 0.25 μm film thickness column purchased from Agilent Technologies (Wilmington, DE). Injection port temperature was set at 220 °C with a split ratio of 20:1. Injection volume of 1 μl was used for all samples. Helium was used as the carrier gas and flow was a constant 2.0 ml/min. The separation program consisted of an initial oven temperature of 50 °C and a final oven temperature of 185 °C. The ramp rate was 30 °C/min with a 4 min hold at the final temperature. The FID was set at 220 °C, hydrogen flow at 40 ml/min, air flow at 450 ml/min, and column plus makeup helium flow at 50 ml/min. Data were collected using Agilent Chemstation software. Retention times for eluted peaks included: methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitate, and methyl stearate. The area percents were normalized using relative response factors (Perkins, 1993). Single fatty acid content was computed as a percentage of total fatty acids.

Fatty acid analysis method validation: The gas chromatography analysis, extraction, and derivatization procedures were validated for linearity, precision, accuracy, and sample stability. Stock solutions of methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitate, and methyl stearate were made from NuChek Prep (Elysian, MN) standards. Dilutions were made from the stock solutions to yield six point linearity curves ranging from 0.005 to 5.2 mg/ml for each fatty acid

Table 2
Total oil and fatty acid methyl ester contents (%) for 31 accessions of *C. calophylla*

Taxonomic name	Identifier	Total oil	C8	C10	C12	C14	C16	C18:0	C18:1	C18:2	C18:3
C. calophylla	PI 578139	30.83	1.23	28.14	64.66	2.93	0.24	–	0.87	1.91	–
C. calophylla	PI 534782	29.08	4.40	20.17	57.99	6.67	2.12	0.87	3.37	4.42	–
C. calophylla	PI 534780	28.95	4.16	19.33	58.29	7.14	2.30	0.45	3.66	4.67	–
C. calophylla	PI 534781	28.79	2.44	28.77	55.89	4.40	1.50	0.19	2.59	4.23	–
C. calophylla subsp. *calophylla*	PI 578143	31.74	0.95	21.21	[71.78]	4.08	0.30	–	–	1.69	–
C. calophylla subsp. *calophylla*	PI 578144	30.62	4.26	21.05	59.13	5.97	1.84	–	3.37	4.37	–
C. calophylla subsp. *calophylla*	PI 566694	27.79	4.14	21.39	57.61	5.81	1.79	–	3.51	5.75	–
C. calophylla subsp. *mesostemon*	PI 578156	33.88	–	24.67	69.48	2.57	0.28	–	1.25	1.75	–
C. calophylla subsp. *mesostemon*	PI 578145	32.47	0.64	25.63	67.60	2.46	0.87	–	1.23	1.58	–
C. calophylla subsp. *mesostemon*	PI 578163	32.19	1.34	31.02	62.15	2.47	–	–	1.18	1.85	–
C. calophylla subsp. *mesostemon*	PI 578159	32.15	–	24.48	69.58	2.77	0.26	–	1.23	1.69	–
C. calophylla subsp. *mesostemon*	PI 578146	31.76	–	25.24	68.25	2.51	0.85	–	1.33	1.82	–
C. calophylla subsp. *mesostemon*	PI 578157	31.54	–	25.58	69.47	2.36	–	–	1.12	1.47	–
C. calophylla subsp. *mesostemon*	PI 578162	31.19	0.39	27.69	67.24	2.72	–	–	1.00	0.96	–
C. calophylla subsp. *mesostemon*	PI 578151	31.11	1.71	31.68	58.65	3.06	1.05	–	1.29	2.56	–
C. calophylla subsp. *mesostemon*	PI 578148	31.06	0.60	25.32	67.47	2.65	0.89	–	1.37	1.70	–
C. calophylla subsp. *mesostemon*	PI 578165	30.96	0.37	27.73	65.02	3.08	0.40	–	0.90	2.49	–
C. calophylla subsp. *mesostemon*	PI 578147	30.75	–	17.98	69.51	5.22	1.51	–	2.11	3.67	–
C. calophylla subsp. *mesostemon*	PI 578160	30.62	–	25.93	69.25	2.21	–	–	1.13	1.48	–
C. calophylla subsp. *mesostemon*	PI 578155	30.44	1.08	27.79	63.83	2.88	0.87	–	1.67	1.88	–
C. calophylla subsp. *mesostemon*	PI 578164	30.30	–	27.11	68.15	2.34	–	–	1.19	1.21	–
C. calophylla subsp. *mesostemon*	Ames 15482	30.00	0.45	29.91	64.09	2.48	–	–	1.37	1.71	–
C. calophylla subsp. *mesostemon*	PI 596722	29.40	0.83	27.61	64.21	2.81	0.57	–	1.50	2.46	–
C. calophylla subsp. *mesostemon*	PI 578149	28.63	0.41	29.30	62.52	2.86	0.38	–	1.55	2.98	–
C. calophylla subsp. *mesostemon*	PI 578182	28.27	1.43	31.06	59.16	2.68	1.05	–	2.10	2.52	–
C. calophylla subsp. *mesostemon*	PI 578152	28.24	0.80	21.84	70.21	2.66	0.97	–	1.48	2.04	–
C. calophylla subsp. *mesostemon*	PI 534667	27.48	1.42	[32.51]	59.88	2.21	0.63	–	1.31	2.05	–
C. calophylla subsp. *mesostemon*	PI 578150	26.85	0.90	22.78	68.33	2.84	1.06	–	1.65	2.45	–
C. calophylla subsp. *mesostemon*	PI 578153	26.50	1.34	31.64	59.38	2.69	1.04	–	1.66	2.26	–
C. calophylla subsp. *mesostemon*	PI 596721	26.30	4.20	20.21	59.94	6.42	1.91	–	3.17	4.15	–
C. calophylla subsp. *mesostemon*	PI 578158	25.60	0.58	22.64	69.45	2.73	0.98	–	1.61	2.00	–

Bracketed numbers represent the highest recorded methyl caprate and methyl laurate levels for the species.

methyl ester. Because we are interested in identifying lines rich in methyl caprate and methyl laurate, recovery studies were conducted by adding methyl caprate and methyl laurate to an assayed sample. The assayed samples were spiked to contain twice the original amounts of methyl caprate and methyl laurate. Sample preparation precision was tested by preparing six samples of PSR23 seed and calculating the relative standard deviation of methyl caprate and methyl laurate levels. Sample stability was studied by multiple injections over a 24 h time period of a PSR23 sample kept at room temperature.

3. Results and discussion

3.1. Fatty acid methyl ester content by gas chromatography

3.1.1. Linearity

A fatty acid methyl ester gas chromatogram is presented in Fig. 1. Concentrations of methyl caprate and methyl laurate ranged from 0.0052 to 5.2 mg/ml. These concentrations were chosen because they are proportional to the total weight percent of methyl caprate and

Table 3
Total oil and fatty acid methyl ester contents (%) for 40 accessions of *C. tolucana*

Identifier	Total oil	C8	C10	C12	C14	C16	C18:0	C18:1	C18:2	C18:3
PI 534810	38.27	0.65	28.32	59.57	3.69	1.51	0.64	1.64	4.00	–
PI 534794	37.32	0.61	26.71	60.18	4.00	1.67	0.70	1.76	4.37	–
PI 534802	36.47	0.52	37.20	49.86	1.75	1.37	0.23	2.47	6.61	–
PI 534796	36.39	0.56	25.90	60.16	4.24	1.85	0.41	1.98	4.90	–
PI 534812	36.11	0.65	30.33	58.05	3.49	1.42	0.56	1.55	3.95	–
PI 534809	35.54	0.57	26.20	60.92	3.93	1.63	0.53	1.72	4.52	–
PI 534801	35.22	0.52	35.79	49.76	2.02	1.51	0.84	2.77	6.79	–
PI 534803	35.05	0.62	22.04	62.73	5.42	2.07	0.56	2.32	4.24	–
PI 534805	34.93	0.50	37.47	49.61	1.66	1.25	0.62	2.84	6.06	–
PI 534910	34.72	0.73	[41.62]	46.30	1.88	1.23	0.64	2.41	5.19	–
PI 534811	34.63	0.49	25.21	62.11	4.61	1.58	0.54	1.59	3.88	–
PI 534793	34.51	0.57	26.59	59.08	4.22	1.90	0.63	2.05	4.96	–
PI 534791	34.49	0.62	38.55	47.90	1.90	1.41	0.47	3.01	6.14	–
PI 534789	34.48	0.50	35.57	49.89	1.88	1.45	0.90	3.45	6.36	–
PI 534887	34.19	0.54	25.89	62.69	3.69	1.41	0.72	1.19	3.86	–
PI 534723	34.13	0.50	36.05	50.24	1.81	1.37	0.72	2.53	6.78	–
PI 534804	33.87	0.45	33.11	54.23	2.06	1.24	0.60	2.65	5.67	–
PI 534807	33.82	0.48	28.53	59.21	2.87	1.35	0.62	2.27	4.66	–
PI 534797	33.58	0.56	25.91	59.96	4.18	1.84	0.74	1.95	4.84	–
PI 561497	33.58	0.51	25.46	61.58	3.73	1.66	0.70	2.09	4.27	–
PI 534799	33.53	0.54	27.75	59.22	4.63	1.89	0.37	1.70	3.89	–
PI 560078	33.29	0.57	24.52	61.82	4.27	1.80	0.75	1.65	4.63	–
PI 594927	33.14	0.46	24.11	62.00	3.74	1.64	0.69	2.62	4.74	–
PI 560076	33.07	0.40	26.80	61.90	3.81	1.48	0.39	1.29	3.92	–
PI 534886	32.84	0.57	24.23	61.90	4.35	1.84	0.56	1.82	4.73	–
PI 560079	32.51	0.57	24.30	62.01	4.29	1.82	0.70	1.65	4.67	–
PI 534800	32.50	0.51	26.49	59.78	4.81	2.00	0.63	1.77	4.01	–
PI 534725	32.17	0.62	32.97	52.61	3.01	1.67	0.76	2.37	5.98	–
PI 534806	32.04	0.51	26.72	60.86	3.42	1.50	0.58	1.98	4.43	–
PI 534795	31.88	–	21.42	[64.57]	5.38	1.85	0.60	1.80	4.37	–
PI 534724	31.68	0.44	34.37	51.19	1.80	1.42	0.72	2.87	7.18	–
PI 534808	31.66	0.54	26.24	61.74	3.58	1.44	0.40	1.98	4.09	–
PI 561496	31.66	0.52	27.68	57.89	4.51	1.99	0.70	1.90	4.81	–
PI 561498	31.19	0.47	22.35	64.19	3.98	1.66	0.58	2.16	4.62	–
PI 594926	31.04	0.52	33.31	50.61	1.86	1.57	0.80	4.14	7.18	–
PI 534798	30.98	0.30	35.12	49.94	1.79	1.51	0.96	3.44	6.94	–
PI 534792	30.64	0.58	34.89	51.30	2.52	1.52	0.67	2.72	5.81	–
PI 534722	30.34	–	34.81	53.18	1.12	0.92	–	2.85	7.12	–
PI 534813	29.71	0.65	27.81	58.39	3.97	1.83	0.51	2.12	4.72	–
PI 560077	29.66	0.41	26.99	60.03	4.03	1.80	0.16	2.05	4.54	–

Bracketed numbers represent the highest recorded methyl caprate and methyl laurate levels for the species.

methyl laurate in the seed ranging from 0.04% to 40%. Linearity curves were also generated for methyl caprylate, methyl myristate, methyl palmitate, and methyl stearate. All curves fell within a 99.998% confidence level. Relative response factors for the fatty acid methyl esters agreed with those reported by Perkins (1993).

3.1.2. Accuracy and precision

Weight percent methyl caprate and methyl laurate were calculated using the gas chromatography response factors. The recovery rates for methyl caprate and methyl laurate were 98%. Sample preparation precision was measured by calculating relative standard deviations of the methyl caprate and methyl laurate results from one accession prepared and analyzed six times. The relative standard deviations were 2.9% for methyl caprate and 6.1% for methyl laurate.

3.1.3. Sample stability

The Cuphea seed oil extract remained stable over a 24 h period at room temperature. No changes in the oil constituents were observed during the testing period.

3.1.4. Fatty acid methyl ester content for 185 accessions

The fatty acid methyl ester and total oil results from the 60 accessions of *C. lanceolata* assayed are listed in Table 1. Weight percent triglycerides ranged from 20.8% to 33.3%. *C. lanceolata* is rich in methyl caprate; and levels ranged from 77.8% to 91.4%. Methyl laurate levels ranged from 1.2% to 4.3%. Accession PI 594936 contained the highest level of total oil with 33.3%, while PI 534853 contained the highest level of methyl caprate at 91.4%. The highest level of methyl laurate was found in PI 561486.

Table 4
Total oil and fatty acid methyl ester contents (%) for accessions rich in methyl laurate

Taxonomic name	Identifier	Total oil	C8	C10	C12	C14	C16	C18:0	C18:1	C18:2	C18:3
C. carthagenensis	PI 534673	29.35	2.62	14.98	[67.50]	8.06	1.76	–	1.67	3.41	–
C. carthagenensis	PI 578172	28.58	4.03	20.11	58.93	6.19	1.88	–	3.73	5.14	–
C. carthagenensis	PI 560080	28.44	2.15	8.70	67.31	11.68	2.49	0.28	2.62	4.77	–
C. carthagenensis	PI 534674	27.37	2.81	11.44	62.87	12.77	2.59	0.19	3.31	4.01	–
C. glossostoma	PI 534841	31.22	0.77	32.09	[57.73]	3.95	1.24	–	1.13	3.09	–
C. glutinosa	PI 534680	23.37	5.75	23.50	[54.22]	5.32	1.95	0.72	2.24	6.29	–
C. heterophylla	PI 596717	29.02	2.74	[42.16]	41.55	4.36	1.54	0.60	2.80	4.24	–
C. laminuligera	PI 561484	33.44	0.69	24.69	59.96	5.45	2.09	0.57	1.90	4.65	–
C. laminuligera	PI 561482	32.12	0.79	30.18	59.55	4.10	1.37	–	1.08	2.94	–
C. laminuligera	PI 561483	31.96	0.58	24.64	[60.07]	5.52	2.00	0.58	1.88	4.73	–
C. laminuligera	PI 534900	30.50	1.10	36.86	52.45	3.56	1.29	0.36	1.11	3.27	–
C. lutea	PI 607957	32.20	1.96	37.92	36.18	9.40	2.72	0.65	5.87	5.31	–
C. lutea	PI 534903	30.99	1.72	35.45	[41.89]	8.50	2.26	0.72	5.10	4.35	–
C. lutea	PI 578184	30.68	2.27	[39.06]	36.03	9.31	2.52	0.45	5.03	5.31	–
C. lutea	Ames 17799	30.58	1.87	33.63	37.86	10.95	2.97	1.22	7.11	4.39	–
C. lutea	PI 534902	29.95	1.76	32.62	38.98	10.31	2.98	0.66	6.51	6.18	–
C. lutea	PI 578176	29.71	1.63	31.73	39.62	10.54	3.03	0.70	6.60	6.14	–
C. lutea	PI 578185	29.67	2.30	33.01	34.43	13.16	3.66	1.01	6.73	5.69	–
C. lutea	PI 578177	29.37	1.73	33.09	34.50	11.58	3.46	0.95	7.76	6.92	–
C. lutea	PI 578183	28.73	1.99	34.68	35.68	11.10	3.11	1.09	6.54	5.82	–
C. lutea	Ames 17815	28.56	1.86	[39.06]	37.06	8.10	2.37	0.85	5.46	5.24	–
C. lutea	PI 578186	28.51	2.37	38.65	35.94	9.53	2.66	0.27	5.70	4.89	–
C. lutea	PI 578187	27.64	2.37	38.89	35.12	9.31	2.56	0.72	5.99	5.03	–
C. lutea	PI 534873	27.52	1.57	29.76	38.65	11.36	3.43	0.92	7.31	6.99	–
C. lutea	PI 534701	27.43	1.79	33.96	38.99	10.59	2.91	0.83	6.22	4.72	–
C. parsonia	PI 534706	24.50	3.00	14.48	[63.29]	9.14	2.19	–	3.96	3.94	–
C. wrightii var. *wrightii*	PI 561512	39.48	0.35	15.06	[72.84]	4.68	1.47	0.53	1.32	3.75	–
C. wrightii var. *wrightii*	PI 561506	35.07	0.58	26.90	59.90	4.24	1.78	0.52	1.74	4.34	–
C. wrightii var. *wrightii*	PI 561509	33.54	1.07	28.50	58.43	4.35	1.78	0.41	1.66	3.81	–

Bracketed number represents the highest recorded predominant single fatty acid for each species.

The fatty acid methyl ester and total oil content for the 31 accessions of *Cuphea calophylla* are listed in Table 2. Weight percent triglycerides ranged from 25.6% to 33.9%. Methyl caprate levels ranged from 18.0% to 32.5%. *C. calophylla* is rich in methyl laurate; levels ranged from 55.9% to 71.8%. Accession PI 578156 contained the highest level of total oil with 33.9%, while PI 534667 contained the highest level of methyl caprate at 32.5%, and PI 578143 contained the highest level of methyl laurate at 71.8%.

The fatty acid methyl ester and total oil contents of the 40 accessions of *Cuphea tolucana* are listed in Table 3. Weight percent triglycerides ranged from 29.7% to 38.3%. Methyl caprate levels ranged from 21.4% to 41.6%. *C. tolucana* is also rich in methyl laurate where the contents ranged from 46.3% to 64.6%. Accession PI 534810 contained the highest level of total oil with 38.3%, while PI 534910 contained the highest level of methyl caprate at 41.6%, and PI 534795 contained the highest level of methyl laurate at 64.6%.

The results from other species rich in methyl laurate are presented in Table 4. Four accessions of *C. carthagenensis* and *C. laminuligera*, 14 accessions of *C. lutea*, 1 accession of *C. glossostoma*, *C. glutinosa*, *C. heterophylla*, and *C. parsonsia*, and 3 accessions of *Cuphea wrightii* var. *wrightii* were identified as accessions high in methyl laurate. Weight percent triglycerides ranged from 23.4% in *C. glutinosa* to 39.5% in *C. wrightii* var. *wrightii*. Methyl caprate levels ranged from 8.7% in *C. carthagenensis* to 42.2% in *C. heterophylla*. For the 185 accessions, the highest level of methyl laurate was present in *C. wrightii* var. *wrightii.* Methyl laurate levels ranged from 34.4% in *C. lutea* to 72.8% in *C. wrightii* var. *wrightii.*

The results from other species with substantial amounts of methyl caprate are presented in Table 5. Twenty-one accessions of *C. procumbens*, two accessions of *Cuphea llavea*, and one accession of *C. hybrid* and *C. viscosissima* were rich in methyl caprate. Triglycerides ranged from 10.1% in *C. llavea* to 35.3% in *C. procumbens*. For the accessions rich in methyl caprate, *C. viscosissima* (PI 534726) contained the lowest levels of methyl caprate at 72.5%, while *C. llavea* (PI 534698) contained the highest level at 92.0%. *C. hybrid* contained a methyl caprate level of 85.1%. The highest level of methyl caprate in *C. procumbens* was found in acces-

Table 5
Total oil and fatty acid methyl ester content (%) for accessions rich in methyl caprate

Taxonomic name	Identifier	Total oil	C8	C10	C12	C14	C16	C18:0	C18:1	C18:2	C18:3
C. sp. hybrid	PI 534785	26.61	0.78	[85.06]	2.26	1.77	2.70	0.79	2.58	4.06	–
C. llavea	PI 534698	34.42	1.68	[92.03]	1.19	0.34	1.18	–	1.16	2.42	–
C. llavea	PI 607969	10.10	–	82.68	2.54	1.10	3.35	0.73	4.56	5.04	–
C. procumbens	PI 534901	35.25	0.63	84.45	1.56	1.19	2.58	0.72	5.00	3.87	–
C. procumbens	PI 534881	34.85	1.14	87.45	1.40	1.22	1.97	1.28	2.98	2.58	–
C. procumbens	PI 560083	34.30	1.31	86.22	1.53	1.17	2.17	0.69	3.66	3.23	–
C. procumbens	PI 534883	33.61	1.23	84.98	1.51	1.63	2.74	0.92	4.02	2.97	–
C. procumbens	PI 534711	33.48	1.21	88.96	1.39	1.05	1.68	0.67	2.66	2.37	–
C. procumbens	PI 534788	32.72	0.90	86.47	1.53	0.79	1.91	0.97	3.97	3.38	0.09
C. procumbens	PI 534832	32.22	1.14	88.39	1.42	1.22	1.77	0.94	2.99	2.13	–
C. procumbens	PI 534709	30.97	0.99	86.89	1.54	1.08	2.00	0.84	3.80	2.86	–
C. procumbens	PI 534710	30.88	1.07	88.65	1.60	1.10	1.74	0.71	2.37	2.76	–
C. procumbens	PI 534712	30.76	1.17	88.01	1.60	1.10	1.83	0.91	2.88	2.50	–
C. procumbens	PI 534908	30.21	0.59	85.05	1.45	1.03	2.22	0.99	5.26	3.42	–
C. procumbens	PI 534878	30.03	1.05	88.61	1.43	0.93	1.63	0.71	3.06	2.58	–
C. procumbens	PI 534713	29.36	1.09	85.55	1.73	1.44	2.47	0.55	3.91	3.26	–
C. procumbens	PI 534880	29.18	1.37	[89.24]	1.36	1.03	1.65	0.76	2.41	2.18	–
C. procumbens	PI 534885	29.09	1.10	84.58	1.42	0.53	2.28	1.62	4.43	4.03	–
C. procumbens	PI 594959	28.99	1.05	86.36	1.45	1.47	2.25	1.20	3.67	2.54	–
C. procumbens	PI 534714	28.19	1.05	83.50	1.95	1.73	2.81	1.21	3.64	4.10	–
C. procumbens	PI 534708	25.36	1.18	85.96	1.92	1.48	2.42	0.32	3.46	3.26	–
C. procumbens	PI 534787	25.36	0.93	83.63	1.57	0.93	2.38	1.36	4.90	4.18	0.12
C. procumbens	PI 534879	19.28	0.55	85.76	2.31	1.70	2.47	0.60	2.34	4.28	–
C. procumbens	PI 561492	19.23	0.99	81.08	1.88	1.66	3.26	1.75	3.96	5.41	–
C. viscosissima	PI 534726	29.89	17.13	[72.48]	2.63	0.78	1.65	0.57	1.63	3.13	–

Bracketed number represents the highest recorded predominant single fatty acid for each species.

sion PI 534880 at 89.2%. Methyl laurate levels ranged from 1.2% in *C. llavea* to 2.6% in *C. procumbens.*

4. Conclusions

Cuphea seeds were analyzed for total seed oil content by pulsed NMR and fatty acid methyl ester constituents by gas chromatography. The gas chromatographic method was validated for accuracy and precision of methyl caprate and methyl laurate as well as linearity and prepared sample stability. The relative response factors of the fatty acid methyl esters agreed with previously published results. *C. wrightii* var. *wrightii* was identified with high seed oil content and as a rich in methyl laurate. *C. llavea* was rich in methyl caprate. Species and specific accessions of Cuphea identified here as having high seed oil content and a high percentage of single fatty acid methyl esters can now be utilized in current breeding programs to further the development of Cuphea as a viable new crop for U.S. agriculture.

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